

AMENDMENTS TO THE SPECIFICATION

In the specification at page 3, line 25, please replace the paragraph which starts with “(ii) Addition of inhibitors” with the following amended paragraph:

(ii) Addition of inhibitors of the enzyme phytoene desaturase, such as, for example, diphenylamine and cinnamyl alcohol, can block further conversion of phytoene, causing the latter to accumulate (Cerdá-Olmedo, 1989, In: E. Vandamme, ed. Biotechnology of vitamin, growth factor and pigment production. London: ~~Elsevier~~ Eisevier Applied Science, pp. 27-42).

In the specification at page 18, line 2, please replace the paragraph which starts with “The vector employed” with the following amended paragraph:

The vector employed comprises genetic information or parts of said genetic information for producing carotenoids or their precursors, in particular carotenes or xanthophylls or their precursors. The vector employed comprises preferably genetic information for producing astaxanthin, zeaxanthin, echinenone, β -cryptoxanthin, β -carotene, andonixanthin, adonirubine, canthaxanthin, 3-hydroxyechinenone, 3'-hydroxyechinenone, lycopene, lutein, phytofluene, bixin or phytoene. Very particularly preferably, the vector comprises information for producing bixin, phytoene, canthaxanthin, astaxanthin or zeaxanthin.

In the specification at page 21, line 7, please replace the paragraph which starts with “Reduced regulation” with the following amended paragraph:

Reduced regulation may preferably ~~also~~ be achieved by a promoter which is functionally linked to the coding sequence in the nucleic acid construct and which is subject to a reduced regulation in the organism, compared with the wild type promoter.

In the specification at page 48, line 12, please replace the paragraph which starts with “By combining according” with the following amended paragraph:

By combining according to the invention the production of two products, in particular in the preparation according to variant a), namely the at least one carotenoid and the carotenoid-containing foodstuff, there is no need for complete extraction of the carotenoids from the biomass so that said extraction is less complicated. Despite complete utilization, the carotenoid needs to be extracted only partially, and no product is lost. This requires ~~small~~ smaller amounts of solvent, accompanied by fewer measures for their reuse. Moreover, waste products are largely avoided, since the biomass does not end up as waste but is processed further to give a foodstuff of high value. As a result, the methods become less expensive due to the utilization of synergies.

In the specification at page 68, line 8, please replace the paragraph which starts with "To select for" with the following amended paragraph:

To select for transformed *Blakeslea* cells, the medium contained hygromycin at a concentration of 100 mg/l and, to select against agrobacteria, 100 mg/l cefotaxime. The incubation was carried out at 26°C for approx. 7 days. This was followed by transferring mycelium to fresh selection plates. Resultant spores were rinsed with 0.9% NaCl and plated on CM17-1 agar (3 g/l glucose, 200 mg/l L-asparagine, 50 mg/l MgSO₄ x 7H₂O, 150 mg/l KH₂PO₄, 25 µg/l thiamine-HCl, 100 mg/l Yeast Extract, 100 mg/l sodium deoxycholate, pH 5.5, 100 ~~mg/L~~ mg/l cefotaxime, 100 ~~mg/L~~ mg/l hygromycine, 18 g/l agar). The transfer of spores to fresh selection plates was repeated three times. In this way, the transformant *Blakeslea trispora* GMO 3005 was isolated. Alternatively, the GMO (genetically modified organisms) were selected by applying the spores individually to CM-17 agar containing 100 mg/l cefotaxime, 100 mg/l hygromycin, by means of the Becton Dickinson FacsVantage+Diva Option. In this case, fungal mycelium formed only where the spores had been genetically modified.

In the specification at page 76, lines 8-22, please replace the section which starts with "p-carRA-HPcrtZ-GCG-3'carA-IR" with the following amended section:

- p-carRA-HPcrtZ-GCG-3'carA-IR, comprising the gene of the HPcrtZ hydroxylase from *Haematococcus pluvialis* Flotow NIES-144 under the control of the *Blakeslea trispora* pcarRA promoter. The hydroxylase gene is fused to an inverted repeat structure which is derived from the 3' end of ~~earRA~~ carA and the region downstream of carA (IR,

SEQ ID NO:74, “Inverted Repeat 1” approx. 350 bp of carA, then approx. 200 bp “Loop” and then approx. 350 bp “Inverted Repeat 2”). Consequently, the derived fusion protein consists of the *Haematococcus pluvialis* hydroxylase and the carboxy terminus of *Blakeslea trispora* CarA (Seq. pBinAHyg-BTpcarRA-HPcrtZ-GCG-3’carA-IR, SEQ ID NO:41, Fig. 9).

In the specification at page 77, lines 3-10, please replace the section which starts with “p-gpdA-HPcrtZ-t-crtZ” with the following amended section:

- p-gpdA-HPcrtZ-t-crtZ, comprising the gene of the HPcrtZ hydroxylase from *Haematococcus pluvialis* Flotow NIES-144 under the control of the gpdA promoter and the t-crtZ terminator; i.e. of the sequence section downstream of crtZ from *Haematococcus pluvialis* Flotow NIES-144 (SEQ ID NO:73) (Seq. pBinAHyg-gpdA-HPcrtZ-tcrtZ, SEQ ID NO:43 ~~NO:43~~ NO:45, Fig. 13).

In the specification at page 79, line 13, please replace the paragraph which starts with “In the inverted” with the following amended paragraph:

In the inverted nested PCR of 200 ng of ~~XhoI~~ XhoI-cleaved and circularized genomic DNA of *Blakeslea trispora* ATCC14272, a 3000-bp fragment was obtained in the following reaction mixture: template DNA (1 µg of genomic DNA of *Blakeslea trispora* ATCC 14272) primers MAT344 5’-GGCGTACTTGAAGGAACCCTTACCG-3’ (SEQ ID NO:63) and MAT 345 5’-ATTGATGCTCCCGGTCACCGTGATT-3’ (SEQ ID NO: 64), 0.25 µM each, 100 µM dNTP, 10 µl of Herculanase polymerase buffer 10×, 5 U of Herculanase (addition at 85°C), H₂O ad 100 µl. The PCR profile was as follows: 95°C, 10 min (1 cycle); 85°C, 5 min (1 cycle); 60°C, 30 s, 72°C, 60 s, 95°C, 30 s (30 cycles); 72°C, 10 min (1 cycle). The sequence section upstream of the putative start codon of the tef1 gene in the 3000-bp fragment was referred to as ptef1 promoter.

In the specification at page 80, line 20, please replace the paragraph which starts with “This DNA probe” with the following amended paragraph:

This DNA probe was used for ~~serreeing~~ screening the cosmid gene library. A clone whose cosmid hybridized with said DNA probe was identified. The insert of this cosmid was sequenced. The DNA sequence comprised a section which was assigned to the gene of an MHG-CoA reductase [HMG-CoA-Red.gb].

In the specification at page 97, line 18, please replace the paragraph which starts with "This DNA probe" with the following amended paragraph:

The removed, dichloromethane-wet biomass, after steam distillation, was spray-dried ($T_i = 125^{\circ}\text{C}$, $T_E = 60^{\circ}\text{C}$) ($T_E = 125^{\circ}\text{C}$, $T_A = 60^{\circ}\text{C}$) and may be used as animal feed supplement.